THE STRUCTURE OF GALLS FORMED BY *CYTTARIA SEPTENTRIONALIS* ON *FAGUS MOOREI*.

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(Plates i–ii; twelve Text-figures.)

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The parasitic fungus *Cyttaria* has been found attacking different species of *Fagus* in South America, Australia and New Zealand. Two species have been recorded in Australia, *Cyttaria Gunnii* Berk., which grows on *Fagus Cunninghami* Hook. in Victoria and Tasmania, and *Cyttaria septentrionalis* Herb. on *Fagus Moorei* F.v.M. in New South Wales and southern Queensland. *C. septentrionalis* was first described by Herbert (1932) from the MacPherson Ranges, on the southern Queensland border, and was later recorded by the writer (1935) from Barrington Tops, Mt. Royal Ranges, north-west of Newcastle, N.S.W.

Cyttaria has been placed in the family Cyttariaceae, an inoperculate family of the Pezizales.

Materials.

The material used in this investigation was collected on 28th August and 6th October, 1935, near the summit of Barrington Tops, New South Wales. Microtome sections of the gall were stained by the iron-alum haematoxylin method, and with gentian violet and orange G. These showed the details of the mycelium. Hand sections were also made and stained with lacto-phenol-cotton blue. By this method the mycelium and cytoplasm stained a bright blue and were differentiated from the host cells. The distribution of the fungus in the tissues could thus be traced.

Gall Formation.

Infection by the fungus causes certain modifications of the host which result in the formation of hard woody galls. Galls develop on all infected stems and branches which are undergoing secondary thickening. Secondary tissues only are infected.

Macroscopic Examination of Galls.

The galls vary from about half an inch to a few feet in length, and from half an inch to about eighteen inches in diameter. They may be long and narrow (Plate i, figs. 1, 2) or short and round (Plate i, fig. 3). Long narrow galls are the commoner, and their shape is due to the fact that infecting mycelium spreads along the cambium chiefly in one direction, parallel to the long axis of the stem. It extends further each year, so that the galls are widest in the centre, tapering off towards each end. The long narrow galls are often somewhat twisted round the stem, following the natural twist of the grain of the wood. In the round short galls the parasitic mycelium has not travelled longitudinally to any extent from the centre of infection.

A transverse section across a gall shows that all the tissues of the stem are not invaded (Text-fig. 1). One or more irregularly wedge-shaped areas of infected tissue can be seen in the stem (A in Text-fig. 1*d*) extending from the cortex nearly to the pith. Each infected section of the stem is generally the result of one primary infection, but compound galls, which owe their origin to two or more primary infections close together, are not uncommon. This condition is shown by the gall illustrated in Plate i, figs. 4*a* and 4*b*. This gall has four components which can be seen externally at A, B, C and D as erumpent areas separated by normal bark. The internal extent of the infected tissues is shown in Text-figure 1, *a–h*, representing transverse sections of the gall taken at intervals of one inch. Infected tissues are shaded, the unshaded parts representing normal xylem. The centre of the stem is marked in each case by a small circle. It can be seen that each infected area may be split up by narrow bands of normal xylem (A in Text-fig. 1*a*), but all are the result of a single infection.

Usually the infected area or areas are on one side of the stem only, giving it a very asymmetrical appearance. This is because infection causes an increase in the size of the tissues near, but not in, these infected areas, making the wood some distance from it on the infected side of the stem much thicker than on the uninfected side (Text-figs. 1 and 2). The twisted appearance of some galls is due to the occurrence of several infections fairly close together.

Age of Galls.

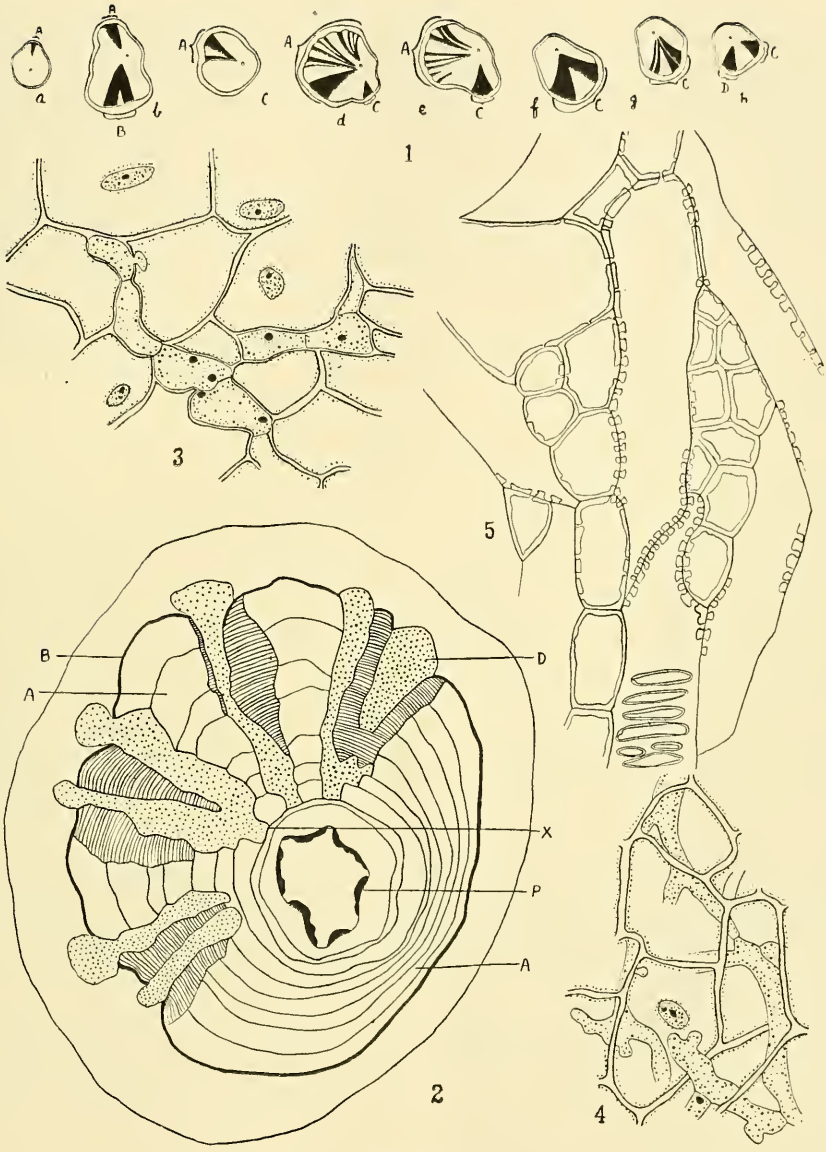
The mycelium is perennial and grows each year during the most active growth period of the host tree. The annual rings are fairly well marked in the uninfected wood of the gall (Plate i, fig. 5). Large vessels are formed each spring, but at the end of the active period of growth thicker-walled tracheids and fibres are formed. The age of any twig or branch can therefore be calculated. By making transverse sections of a gall, a point can be found where the infected tissue most closely approaches the pith. This has been taken to be the point at which infection first took place. It has always been found that infected xylem is present in the second annual ring, indicating that the fungus first becomes active at the commencement of the second growing season. By tracing the inward extent of the fungus in sections progressively nearer the ends of the gall, a region can be found where the infected tissue extends only to the beginning of the third annual ring (X in Text-fig. 2). The distance between this and the area of initial infection gives the rate of growth of the fungus longitudinally along the cambium in one year. Similarly the growth rate in subsequent years can be found. It was found that the growth rate of the fungus in the stem varies considerably from a few millimetres to over 1 centimetre per year.

Tissues Infected.

The tissues susceptible to infection are the cortex, phloem, cambium and secondary xylem. Of these the xylem is the chief tissue infected and forms the bulk of the gall.

(A). *The Secondary Xylem.*—Three types of cells occur in the secondary xylem of the gall: (1) Normal xylem elements; (2) Cells which contain the fungal hyphae; and (3) Cells which do not contain hyphae, but are modified in such a way that they do not develop normally.

(1). Normal xylem consists of vessels, tracheids, fibres and a little parenchyma, interrupted at intervals by xylem rays one or two cells wide and about twelve cells deep (Plate i, figs. 5, 6, 7). Fairly well defined annual rings



Text-figs. 1-5.

1.—Series of transverse sections one inch apart from the compound gall shown in Plate i, figs. 4*a* and 4*b*, to show the areas of the stem occupied by the various components of the gall. Infected areas are shaded and the centre of the stem is marked by a small circle. The various components are shown at A, B, C and D. × 0·5.

2.—Transverse section of a gall near the centre of infection. A, normal xylem; B, cambium; C, infected tissue; P, primary xylem; X, point at which infection extends to third annual ring. × 12.

3-4.—Sections of infected cells showing mycelium. × 720.

5.—Transverse section of portion of a gall showing tracheidal cells. × 720.

are shown (A in Plate i, fig. 5), since there is a definite period of rapid growth each spring following a period of inactivity of the cambium during the winter. These cells in themselves are quite normal, but between infected areas, and for a short distance on either side of infected areas, they are produced in greater numbers than in other parts of the stem (Text-fig. 2), thus giving the increased diameter referred to above.

(2). The tissues containing fungal mycelium resemble ordinary parenchyma. The cells are isodiametric, with fairly thick, but not lignified, walls and they show no prominent pitting (Text-figs. 3, 4; Pl. i, fig. 5; Pl. ii, figs. 8, 16). These cells originate as xylem elements. They become infected with mycelium as they are cut off from the cambium and their normal process of development is modified by the presence of the fungus. Instead of acquiring lignified walls and losing their contents and so becoming vessels, fibres or tracheids, or developing into parenchyma or ray cells, they elongate slightly, but otherwise remain little altered.

(3). The mycelium is not itself found in any other type of cell, but its presence causes modifications in the adjoining xylem (B in Text-fig. 2). These modifications become more marked as the gall increases in age. Young xylem elements in the vicinity of infected cells develop into tracheid-like cells. In an old gall these cells often occupy a larger area than do the infected cells, and it is to them that the gall owes much of its increase in size over that of the stem (Text-fig. 2). In the mature state these cells vary much in size and shape (Text-figs. 5 and 12). The modified cells are usually several times longer than broad (Plate ii, fig. 8). Plate ii, fig. 8 shows infected cells (A) bordered by modified xylem (B) and finally unmodified xylem (C). Plate ii, fig. 12, shows tracheidal cells at the upper edge of an infected area bordered on both sides by normal xylem. These tracheidal cells tend to dove-tail into one another. This is shown especially well in tangential section (Pl. ii, fig. 9) and in transverse section (Text-fig. 5). Their walls are lignified and show prominent scalariform pits with very narrow borders (Pl. ii, fig. 10 and Text-fig. 5). The direction of growth of the tracheidal cells varies considerably as is shown in transverse section (Pl. ii, figs. 10, 11, 8, 12) and longitudinal section (Pl. ii, fig. 9). In these sections cells are seen both longitudinally and transversely arranged. The radial arrangement of the xylem is therefore entirely lost in the region where they occur, and it becomes more irregular the older the gall (Pl. ii, fig. 13).

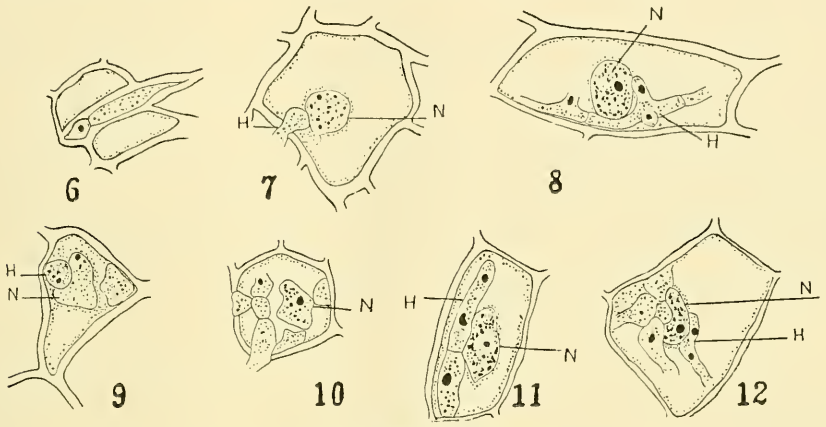
Starch grains are present in great abundance in some of the young tracheidal cells (Pl. ii, fig. 14).

Areas of uninfected xylem are often seen arising in an area of infected xylem (Text-figs. 1*b*, 1*d*, A in Plate i, fig. 5). These are mostly wedge-shaped with the thin edge inward. Each must have originated from a cell of the cambium in the infected region which by chance was uninfected and therefore able to give rise to uninfected cells.

(B). *The Cortex and Phloem.*—In the primary cortex and phloem, infection produces a result resembling in some respects that produced in the xylem. The cells which contain the mycelium are similar in all respects to the infected cells in the xylem. The reaction of the phloem and cortex to fungal invasion differs from that of the xylem principally in that uninfected cells are in no way modified. Infection of the phloem causes an increase in the number of normal cells in the neighbourhood of the infected cells, thus increasing the size of the phloem tissue (Plate i, fig. 7).

The secondary cortex is lacking or only a few cells in width, and appears never to be infected (Plate ii, fig. 15).

(C). *The Cambium.*—The infected cells in the cambium are similar to infected cells in other tissues (B, Plate i, fig. 5). Though the cambium seems to be the centre from which other tissues are infected, the mycelium does not spread in a lateral direction along it further than it does in the xylem or the phloem, nor does it cause any modification of neighbouring cambial cells. Modified tracheidal cells are derived from uninfected cambium which at the same time produces uninfected phloem on the other side. In this case the phloem cells are usually produced at a more rapid rate than in uninfected stems.



Text-figs. 6-12.

6.—Section of an infected cell showing intercellular mycelium. × 960.

7-12.—Sections of infected cells showing effects of haustoria (H) on host nuclei (N). × 960.

The Mycelium within the Gall.

The vegetative mycelium of *Cyttaria septentrionalis* is fairly evenly distributed throughout the tissues it invades, except just below fruiting bodies, where the host cells are more or less completely filled with mycelium. Plate i, fig. 5, shows that no massing of fungal mycelium occurs in the wood.

The mycelium is septate and moderately thin-walled (Text-fig. 3), but the cells vary considerably in length. They usually appear to be uninucleate. This condition does not always obtain in the haustoria, which frequently show the presence of 2 or 3 nuclei (Text-figs. 8, 11, 12). The protoplasm is homogeneous and not very dense (Text-fig. 3).

The mycelium seems to be able to make its way either between the cells or across them, i.e., it is both intra- and inter-cellular (Text-figs. 4, 6). At the point where it enters the cell through the wall it may show a slight constriction (Text-fig. 7), but this is not invariable (see also Text-fig. 4). The intercellular mycelium sends into the cells haustoria which are irregular in shape and often prominently lobed (Text-figs. 9 and 10).

The Effects of the Mycelium on Host Cells and Tissues.

The hypha or haustorium, having entered the cell, usually approaches the nucleus (Text-fig. 4) and finally comes into contact with it (Text-fig. 7), or coils

partially round it (Text-fig. 8). This causes, in most cases, considerable enlargement of the host nucleus. Sometimes a definite change in the shape of the nucleus is apparent; it may become elongated, lobed or kidney-shaped (Text-figs. 9, 11, 12). The fungus does not appear to destroy the nucleus of the infected xylem or phloem cells, and, as far as has been observed, the host cells of these tissues are not eventually killed. Just below a fruiting body, however, the cortical cells become so filled with mycelium that the nucleus and all the contents are completely absorbed and replaced by the fungal mycelium.

The result of infection on the tissues as a whole is a general enlargement of part of the stem, i.e., the formation of a hyperplastic gall, which is due to increase in the number of the cells and not to increase in size of the existing cells (i.e., hypertrophy).

The greatest increase takes place in the xylem and phloem, the primary cortex seldom being heavily infected. Text-figure 2 shows the normal proportion of infection in each tissue.

The increased rate of cell production in the phloem causes the bark covering the gall outside an infected area to become thicker than outside normal wood (Plate ii, figs. 15, 16), even when it contains no mycelium. It is, however, frequently ruptured by the rapid expansion of the tissues beneath it, and, in addition, shows various scars left by the fruiting bodies of previous years. The phellogen is a very narrow band and is lacking over the ruptured areas.

Infection does not seem to cause the death of a tissue.

The Effect of Gall Formation on the Growth of Fagus.

The formation of galls on the branches of *Fagus* seldom seems to do the tree serious injury. Since no tissues are killed and since, in most cases, there is a considerable part of the stem at the level of the gall which contains normal tissues, the passage of food materials and water up and down the stem is not unduly restricted. Very large and apparently healthy trees were observed to be heavily covered with galls (Plate i, fig. 1). In one case a large gall was observed on the main trunk of a tall living tree within a few feet of the ground.

Suggested Means of Infection.

A macroscopic examination shows that large branches have only old galls, never young ones. The young galls are found only on young stems, indicating that primary infection takes place only when the stem is young. It would be impossible for mycelium to penetrate the hard bark of an old stem. If an invading hypha entered through a lenticel, it would still have to cross the cortex, in which there are one to several bands of stone cells, and the phloem before it could infect the cambium, which has been shown to be the centre of infection in the gall.

There is no trace of fungal mycelium in the primary xylem or pith. In the galls examined the first trace of infection occurs in the xylem and phloem of the second year's growth. These observations suggest the following hypothesis as to how infection may take place. During the late spring and early summer, October to early December at Barrington Tops, the spores of *Cyttaria* mature and are blown through the air in great numbers. At the same time the young shoots of *Fagus* are elongating and are still covered with a somewhat hairy epidermis. Secondary thickening commences in these young shoots towards the end of the growing season. The spore, alighting on the epidermis of the young shoots, germinates and the germ tube penetrates the epidermis and the cortex. The

mycelium then probably remains dormant until the beginning of the next spring, either in the cortex or in one of the medullary rays, or, most probably, in the young cambium. When secondary growth begins in the following year, it infects the young xylem and phloem cells as they are developing, and this process goes on yearly. The mycelium also infects the cambium in a longitudinal direction.

Summary.

Cyttaria septentrionalis Herb. is a parasitic fungus which infects the stems of *Fagus Moorei* in New South Wales.

Infection results in the formation of galls very varied in shape and size.

Wedge-shaped areas of infection occur in the stem. Usually one side of the stem is not affected, but contains normal tissue. A gall may be the result of one or more infections and thus may be called simple or compound.

The age and growth-rate of an infected area can be calculated by observing its relationship to the annual rings of the stem.

The tissues infected are the primary cortex, secondary phloem, cambium, and secondary xylem. The xylem contains three groups of cells, normal elements, parenchymatous cells containing mycelium, and tracheidal cells, containing no mycelium but modified as a result of the infection of the neighbouring cells. Starch is present in the young tracheidal cells.

The cambium, phloem and primary cortex consist only of normal cells and parenchymatous cells containing mycelium. A smaller area in the cortex is infected than in the xylem, but in old galls the increase in phloem tissue is proportionate to that in the xylem.

The mycelium is septate, thin walled and 1- to 3-nucleate. It is both inter- and intra-cellular, and produces irregularly-shaped haustoria. It is distributed evenly throughout the tissue it invades, except just below the fruiting bodies, where it almost completely fills the cells.

The haustorium approaches the nucleus and partially coils round it, causing its enlargement or lobing, though it does not destroy it. The host cells are not killed. In some cases cells appear to arise which are free of infection.

Infection of the stem causes enlargement due to increase in the number of cells. This is most pronounced in the xylem and phloem, very little increase taking place in the other tissues. The bark is thicker outside infected areas because of the increase in the amount of the phloem, and is much ruptured and scarred.

Galls do not appear to cause serious damage to, or restrict the growth of, the trees on which they grow.

Macroscopic and microscopic examinations suggest that the mycelium from the germinating spores enters the young stem during the late spring or early summer, just before secondary thickening begins or while it is taking place. The mycelium then probably remains dormant in or near the cambium until the beginning of the second year's growth. It then proceeds to infect the young xylem and phloem cells and continues to do so from year to year. The mycelium also travels along the cambium in a longitudinal direction.

In conclusion, the writer wishes to thank Assistant Professor J. McLuckie and Miss Lilian Fraser for their interest and helpful suggestions throughout the course of this work.

Literature Cited.

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WILSON, J. M., 1935.—A species of *Cyttaria*, apparently *C. septentrionalis*. PROC. LINN. SOC. N.S.W., lx (5-6), pp. xlii-xliii.

DESCRIPTION OF PLATES I-II.

Plate i.

1.—Small branch of *Fagus Moorei*, showing numerous galls. × 0·07.

2.—Part of a branch of *Fagus Moorei* showing a long, narrow gall. × 0·6.

3.—Part of a branch of *Fagus Moorei* showing a round, short gall. × 0·6.

4*a*, 4*b*.—Two views of a compound gall. The various components of the gall are shown at A, B, C and D. *a*, *b*, *c*, etc., mark the places from which the sections represented diagrammatically in Text-figure 1 were cut. × 0·6.

5.—Portion of a transverse section of a gall showing areas infected by *Cyttaria*. A, wedge-shaped area of uninfected xylem; B, infected cambium; C, annual rings; D, infected xylem. × 37.

6.—Radial longitudinal section of portion of a young stem of *Fagus* showing normal wood structure. × 210.

7.—Tangential longitudinal section of portion of a young stem of *Fagus* showing normal wood structure. × 210.

Plate ii.

8.—Transverse section of portion of a gall. A, infected cells containing mycelium; B, tracheidal cells; C, normal xylem. × 85.

9.—Tangential longitudinal section of part of an old gall showing tracheidal cells. × 31.

10-12.—Transverse sections of parts of galls showing tracheidal cells. 10, × 375; 11, × 85; 12, × 45.

13.—Transverse section of part of an old gall showing the loss of radial arrangement of the xylem. × 45.

14.—Transverse section of part of a gall showing starch grains in the young tracheidal cells. × 45.

15.—Transverse section of part of a normal stem of *Fagus* showing phloem (P) and phelloderm (X). × 85.

16.—Transverse section of infected phloem showing increase in number of cells due to infection. × 85.